The Synthesis of [N-Methyl-11C]Benztropine

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Introduction

There has been considerable interest in mapping the distribution of neuroreceptors in human using the positron emission tomography (PET).

Muscarinic cholinergic receptors in a living human brain using PET may provide valuable information about receptor changes in patients with Huntington's chorea and Alzheimer's dementia. Benztropine is known to be a potent muscarinic acetylcholine antagonists and is used to treat Parkinson's disease because of its ability to inhibit dopamine uptake[1]. Dewey *et al.* have synthesized [N-Methyl-^{11}C]benztropine and shown that [N-Methyl-^{11}C]benztropine accumulates in striatum of baboons and humans[2].

This study was undertaken to evaluate the potential utility of [N-Methyl-^{11}C] benztropine as ligand for muscarinic acetylcholine receptors. In this paper, we describe the study of optimal conditions for routine preparation of [N-Methyl-^{11}C]benztropine and the calculation of the radiation dose by tissue distribution study for future *in vivo* studies in human with PET.

Materials and Method

General

Melting points were determined with a Yanagimoto micro melting points apparatus and are uncorrected. ^{1}H-NMR spectra were measured by a JNM-FX 100 spectrometer with TMS as the internal standard and IR spectra by a JASCO A-202 IR spectrometer. Preparative TLC was performed on silica gel (DC-Fertigplatten Kieselgel 60 F254 (Merck), Art. 5744) using the solvent indicated below. Analytical HPLC was carried out using a normal phase column (ERC-Si-1262 (ERMA), 6 mm i.d.× 100 mm long) with MeCN/0.01M $(NH_4)_2HPO_4$=6/4 (V/V) as the solvent (flow rate : 1.5 mL/min) and preparative HPLC using a normal phase column (YMC-023-5-06 S-5 60A Sil (YMC), 10 mm i.d.× 250 mm long) with MeCN/0.01M $(NH_4)_2HPO_4$=6/4 (V/V) as the solvent (flow rate : 5 mL/min).

Preparation of the starting materials

Benztropine mesylate was purchased from Aldrich Chem. Co. Inc, 2, 2, 2-trichloroethyl chloroformate and zinc powder were from Wako Pure Chem. Ind., Ltd. and hydrogen chloride gas was from Tsurumi Soda Co. Ltd.

Synthesis of norbenztropine : Benztropine mesylate was converted to its free base by an extraction in alkaline solution. To a solution of the benztropine (1.2 g, 3.8 mmol) dissolved in benzene (40 mL), 2, 2, 2-trichloroethyl chloroformate (1.6 g, 7.6 mmol) was added and was heated under refluxed for 2 days. After cooling down to room temperature, ether (30 mL) was added. The organic layer was rinced with 3N HCl (120 mL × 2) and water (120 mL) and evaporated. The residue was purified by preparative TLC ($CHCl_3$/hexane=1/1) to give the adduct (N-(2, 2, 2-trichloro ethoxycarbonyl)-norbenztropine) (1.0 g). Yield : 58 %. m.p.: 120 - 121 °C. IR ($CHCl_3$) : 1700 cm^{-1} (-N-CO-O-). Elemental Anal. : Calcd. for $C_{22}H_{24}NO_3Cl_3$: C=58.93, H=5.16, N=2.99, Cl=22.69. Found : C=58.90, H=5.05, N=2.96, Cl=22.63. To a solution of the adduct (300 mg, 0.64 mmol) dissolved in acetic acid (10 mL), zinc powder (0.75 g, 11 mmol) was added and then the mixture was stirred at room temperature for 12 hr. After the zinc powder was filtered off, the filtrate was made basic with 3N NaOH (50 mL) and extracted with ether (50 mL × 3). The organic layer was evaporated to give norbenztropine (140 mg). Yield : 75 %. ^{1}H-NMR ($CDCl_3$) : 1.00 - 2.32, 3.24 - 3.68 (12H, m), 5.40 (1H, S, -OCHPh2), 7.00-7.42 (10H, m, arom.H). To a solution of the norbenztropine (120 mg, 0.41 mmol) dissolved in dry ether (25 mL), hydrogen chloride gas was introduced. After removal of the ether, norbenztropine hydrochloride (100 mg), white crystal, was obtained. Yield : 73 %. m.p. : 252 - 253 °C. Elemental Anal. : Calcd. for $C_{20}H_{24}NOCl$: C = 72.59, H = 7.39, N = 4.07, Cl = 11.04. Found : C=72.82, H=7.33, N = 4.25, Cl = 10.75.

^{11}C-Labeling

$LiAlH_4$ was purchased from Fluka Chem. Corp. and THF purchased from Wako Chem. Ind. was distilled just before use.

Synthesis of [^{11}C]CH_3I : [^{11}C]CO_2 was produced *via* the $^{14}N(p,\alpha)^{11}C$ reaction by proton bombardment (18 MeV, 10 μA) of a 24 kg/cm^2 N_2 gas target using the CGR-MeV model 680 Cyclotron located at Tohoku University. [^{11}C]CH_3I was synthesized from [^{11}C]CO_2 by semi-automated apparatus [3].

Synthesis of [N-methyl-^{11}C]benztropine : Synthetic scheme for [N-methyl-^{11}C]benztropine is shown in Fig. 1. Norbenztropine (1 mg) was dissolved in MeCN/DMF/DMSO=15/8/2 (V/V) solution (0.5 mL) and cooled in ice-water bath. [^{11}C]CH_3I was transferred by a stream of He gas into the cooled solution and the mixture was heated at 70 °C for 5 min. After removal of unreacted [^{11}C]CH_3I with He stream, the mixture was supplied to a preparative

HPLC. The effluent was monitored by a UV detector (254 nm) as well as an on-line NaI detector coupled to a multichannel analyzer. The collected [^{11}C]benztropine fractions were evaporated to dryness, redissolved in saline and filtered through a membrane filter (0.22 μm) for animal experiments.

Quality control : Radiochemical purity and the specific activity were determined by an analytical HPLC.

Animal experiments

Tissue distribution : A saline solution of [N-methyl-^{11}C]benztropine (0.74 MBq, 20 μCi) was injected through the lateral tail vein into male ddY mice (32 - 37 g). The mice were killed at 5, 10, 30 and 60 min after injection by a cervical dislocation. Tissue samples were dissected and placed in tared vials. Blood was obtained by heart puncture and urine was by sucking in paper. The ^{11}C radioactivity of the tissue samples and the injection standards were measured in an automated gamma counter (AUTO-GAMMA 500C) and the wet tissue were weighed. All data were corrected for decay.

Calculation of radiation dose: The dosimetry of [^{11}C]benztropine was calculated using the Medical International Radiation Dose (MIRD) Comittee's method[4]. The cumulated radioactivity for [^{11}C]benztropine in mice was determined by a trapezoidal integration of the data for the percentage of injected dose per organ. The organ concentration of radioactivity to the whole-body concentration were assumed to be same for mice and human[5,6]. Therefore, the cumulated radioactivity in human was estimated from that in mice and organ masses as follows;

(Cumulated radioactivity)human

$$= (\text{Cumulated radioactivity})_{\text{mice}} \times (M_t/M_i)_{\text{mice}} \times (M_i/M_t)_{\text{human}}$$

The subscripts t and i refer to total body and organ, respectively. The radiation dose was estimated from the summation of the cumulated activity and absorbed fraction in each source organ [4].

Result and Discussion

As for the synthesis of starting material, the obtained norbenztropine was converted to its HCl salt (crystal), which was stable and able to be stored for a long time at room temperature, because norbenztropine didn't crystallize easily and was somewhat less stable.

Effects of the reaction time on the benztropine yield at various temperatures was examined in cold run and the result is shown in Fig. 2. At reaction temperatures below 40 °C, over 10 min of the reaction time was required for getting benztropine quantitatively, while over 70 °C, 1 min of the reaction time was enough. From this result, the optimal reaction condition was a 5 min reaction at 70 °C.

Fig. 3 shows the HPLC profile for the separation of [N-methyl-^{11}C]benztropine. The

retention times for norbenztropine and benztropine were 9 and 12 min, respectively. The separation is enough to get the chemically pure [N-methyl-^{11}C]benztropine though the [^{11}C]labeled compound was eluted shortly after the mass of the starting material. The radiochemical yield was ranged from 43 to 52 % based on the introduced [^{11}C]CH_3I with decay correction. Radiochemical purity was more than 99 %, the synthesis time, including HPLC purification was 60 min and the specific activity was ranged from 9.9 to 19.5 GBq/ μmol (267 - 527 mCi/ μmol) at the end of synthesis. These results are reproducible and show a sufficient multimillicurie amount and purity to allow preparation for routine medical studies.

The result of tissue distribution study is shown in Table 1. [N-methyl-^{11}C]benztropine was highly accumulated in the lung, kidney, liver, pancreas and modelately in the heart, spleen, small intestine. It was cleared quickly from the blood. The accumulations in the heart, lung and kidney decreased with time, while those in the pancreas and small intestine increased. The brain uptake was not so high, but gradually increased for during the period of 60 min.

The radiation dose in human was shown in Table 2. The high dose was estimated at urinary bladder, 30.8 mGy/GBq.

References

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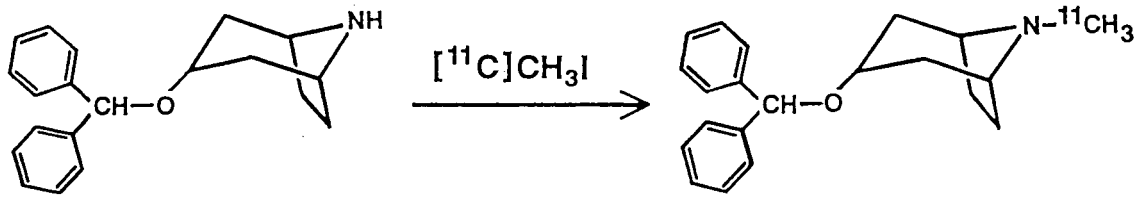
Table 1. Organ Distribution of [N-methyl-^{11}C]Benztropine in Mice[a)]

Organ	Uptake ; DAR[b)]			
	5 min	10 min	30 min	60 min
Brain	0.29 ± 0.05	0.40 ±0.03	0.49 ± 0.05	0.51 ± 0.08
Heart	1.46 ± 0.20	0.99 ± 0.02	0.76 ± 0.13	0.36 ± 0.04
Lung	8.38 ± 1.43	7.73 ± 1.05	3.81 ± 0.79	2.26 ± 0.21
Liver	2.90 ± 0.47	4.14 ± 0.31	3.98 ± 0.36	3.40 ± 0.30
Pancreas	2.66 ± 0.57	4.47 ± 0.32	5.45 ± 1.08	5.36 ± 0.46
Spleen	1.47 ± 0.25	2.21 ± 0.24	2.05 ± 0.28	1.23 ± 0.19
Small Intestine	1.09 ± 0.12	1.59 ± 0.07	2.18 ± 0.23	2.81 ± 0.30
Large Intestine	0.52 ± 0.12	0.69 ± 0.08	0.80 ± 0.14	0.89 ± 0.16
Stomach	0.78 ± 0.17	1.14 ± 0.24	1.54 ± 0.38	1.51 ± 0.22
Kidney	5.66 ± 0.51	6.59 ± 0.23	5.25 ± 0.77	3.15 ± 0.40
Testis	0.13 ± 0.03	0.16 ± 0.03	0.21 ± 0.05	0.24 ± 0.12
Muscle	0.66 ± 0.14	0.61 ± 0.06	0.35 ± 0.07	0.17 ± 0.11
Bone	0.52 ± 0.09	0.68 ± 0.01	0.57 ± 0.27	0.35 ± 0.22
Bladder	0.50 ± 0.09	1.26 ± 1.03	1.21 ± 0.47	1.31 ± 0.34
Blood	0.27 ± 0.05	0.20 ± 0.04	0.24 ± 0.02	0.25 ± 0.06
Urine c)	0.18	0.98	4.34	9.25

a) the mean ± s. d. of four animals
b) DAR = (counts / g tissue) × (g body weight / total injected counts)
c) the percentage of injected dose

Table 2. Estimated Radiation Dose for [N-methyl-^{11}C-Benztropine]

	(Radiation Dose) human mGy / GBq
Brain	0.3
Stomach Wall	2.5
Small Intestine	4.6
Large Intestine	4.9
Kidney	9.7
Liver	7.8
Lung	9.2
Red Marrow	1.2
Pancreas	8.6
Testis	0.9
Bladder Wall	30.8
Total body	1.4



Norbenztropine

[N-methyl-^{11}C]
Benztropine

Fig. 1. Synthetic scheme of [N-methyl-^{11}C]benztropine.

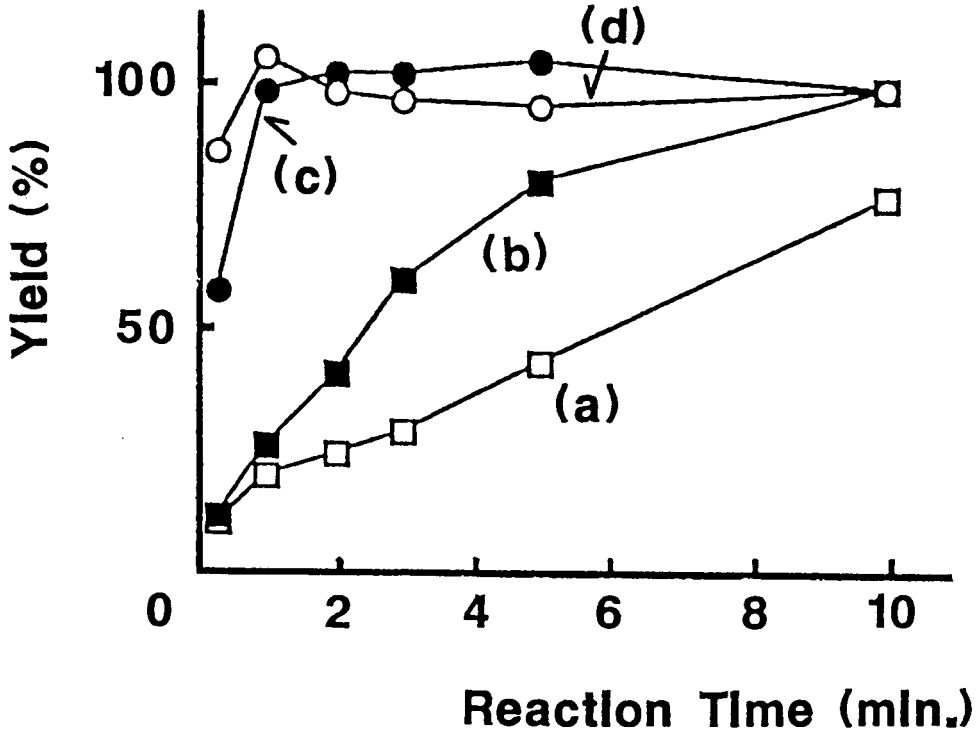


Fig. 2. Effects of the reaction time on the [N-methyl-^{11}C]benztropine yield.
(a) reaction temperature : 24 °C (b) 42 °C (c) 70 °C(d) 130 °C.

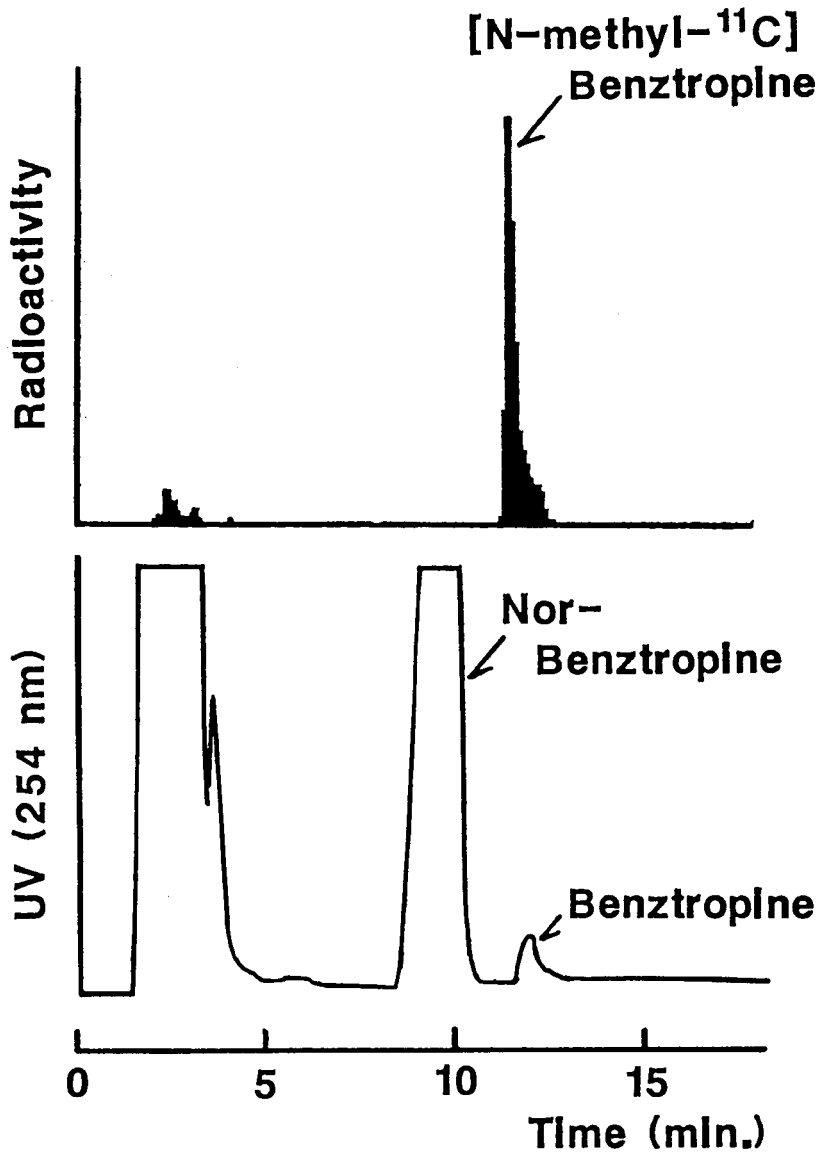


Fig. 3. Chromatographic profile of reaction mixture before HPLC purification.